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Effect of Substrate Types (Saw Dust, Corn Cob) Associated with Insect Activities on the White Oyster Mushroom (*Pleurotus ostreatus*) Yield in Musang-Bamenda III (North West Region-Cameroon)

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ABSTRACT

Oyster mushroom is an edible mushroom which has been identified as an excellent food source to alleviate malnutrition worldwide. To evaluate the effects of different substrate types (saw dust and corn cob) associated with insect activities on the yield of white oyster mushroom, experiments were carried out in exposed house, from the 26th of May to the 31st of August 2022 in Musang. Oyster mushroom was cultivated on different substrates made of six treatments: saw dust (T1, T11), Corn cobs (T2, T22) and a mixture of both (T3, T33), all supplemented with 1% $CaCO_3$. The treatments were tied in black polythene bags each. The experiment was laid in a random design. The different parameters studied show that the highest degree of colonization and the lowest time from primordial initiation to harvest were obtained in T2; The highest biological yield, economic yield and dry weight were observed in T11. The time from primordial initiation to harvest was observed in T1 and the highest average number of fruiting body/packet in T2, the highest average weight of individual fruiting body in T33, the highest mean height of fruiting bodies in T11 and the highest average diameter of Pileus was observed in T22. Pileus, gill and stipe were the different mushroom parts visited by insects belonging to orders Diptera (58.20%), Coleoptera (3.21 %), Blattodea (0.28 %). Insects reduce the quality and quantity of the fungi. Among many aspects, T11 was found as the best substrate with biological and economic yields.

Keywords: Biological yield; Corn cob; Economic yield; Insects; Insect's activities; Musang; Oyster mushroom; Saw dust.

1. Introduction

Oyster mushroom (*Pleurotus ostreatus*) is one kind of edible fungi belonging to the genus Pleurotus under the class Basidiomycetes. It is an edible mushroom having excellent flavor and taste. The species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency [1]. Cultivation of oyster mushroom has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and harvested all over the year [2]. Oyster mushroom is characterized by its rapid growth on agro-wastes such as dried sugar cane leaves, saw dust, maize stover, banana leaves, palm cones, coffee husks and wheat bran which are substrates for mushroom production [3]. These substrate materials can be supplemented with corn flour, rice bran, molasses, soya bean or kernel cake in accordance with the particular substrate material used. Many of mushrooms pose a range of metabolites of intense interest to pharmaceutical (e.g. antitumour, antigenotoxic, antioxidant, anti-inflammatory, anti-hypertensive, anti-platelet aggregating, anti-hyperglycaemic, antimicrobial, antiviral activities) and food industries [4]. In Cameroon, agriculture and forestry are two prominent sectors which bring in revenue to the government [5]. Agricultural development programs must consider all the factors that can lead to increased yields. Among the factors, we consider insects and waste materials. Unfortunately, most of the resulting waste products like sawdust from timber exploitation, rice husk or maize stover, are not often recycled appropriately through their use in the cultivation of mushroom.This may be due to lack of knowledge on the use of these substrate materials in mushroom cultivation [6]. In the other hand, insects serve important ecological functions in a variety of processes,

including nutrient cycling, seed dispersal, bioturbation, pollination, and pest control [7,8]. Fungi and insects are highly diverse groups due to the ability to exploit a wide range of niches [9]. Insects may act as predators while *Pleurotus ostreatus* may be a food/energy resource, nest location for insects [10]. Mushroom-insect interaction was classically viewed as mutualistic, antagonistic, or commensalism [9]. Commercial production of fresh edible mushrooms is a rapidly growing industrial activity that can be carried out in a large or small scale. It is an efficient and relatively short biological process of food protein recovery from negative value lignocellulosic materials, utilizing the degrading capabilities of mushrooms [11]. Strengthening mushroom production sector could be essential in order to enable the rural economy to keep its vibrancy and development, increasing and diversifying business and employment opportunities in the rural areas, and providing income opportunities of small family farms. Malnutrition is a problem in developing third world countries [12]. To alleviate hunger and malnutrition in a world of rising food prices, cultivation of mushrooms is a very reliable and profitable option. Mushroom cultivation can play an important role in managing organic wastes whose disposal has become a problem [13]. These wastes can be recycled into food and environment may be less endangered by pollution [14]. In Cameroon, mushrooms have been and is still being collected in the wild. Wild mushroom has been a delicacy for many years and it is regarded as a meat substitute especially by the rural population [3]. Some mushrooms are edible but others are poisonous and the making being afraid of consuming mushroom [15]. It's practiced is highest in Adamawa Region, followed by West region. With changes of environment, harvesting becomes difficult. Therefore, there is need of growing mushrooms domestically. Some individual farmers and organizations are much involved in the production and transformation of mushroom in Cameroon especially in Bamenda (PDFC, 2012). Most of the research works in Cameroon have been on the taxonomy and documentation of the diversity of macrofungi and very few on modes of cultivation [16, 6].

In order to contribute to the improvement of the yields of this fungi, the present investigations were designed to identify the best substrate composition that promotes the growth of the white oyster and to evaluate the activity of insects visiting the mushroom farm. The information gained will help farmers to develop management plans that could increase the overall quality and quantity of the white oyster mushroom yields in Cameroon.

1.1. Study Objectives

The main objectives of this investigation are: (i) To identify the best substrate composition that promotes the growth of the white oyster. (ii) To evaluate the effect of different substrates on mycelium growth. (iii) To evaluate the effect of different substrate on yield contributing characters. (iv) To evaluate the effect of different substrate on yield. (v) To evaluate the activity of species visiting the mushroom farm and their impact on the production of this fungi.

2. Methods

2.1. Study site

The experimental investigations were carried out from the 26th of May to the 31st of August 2022 in Musang street, in Bamenda 3, North west Region of Cameroon during the rainy season. The study site has coordinates 50561N 10E/5.3933N 10,1670E. Bamenda III council area (22.9 km^2) shows great ecological variation marked by two distinct seasons: a short dry season of 5 months (November to March) and a long rainy season which last for 7

months (April to October). The council area shows a wide variety of relief. Attitudes ranges from 1300 m to 2600 m above sea. Characteristic features include many hills with gentle and steep slopes, and the soil type is sandy loam [17].

2.2. Farm House

The experiment was carried out in exposed house, with open doors. A clean room were the ingredients shielded from environmental factors such as wind, rain, sunlight was use for cultivation. It was well-spaced out with shelves with well cemented floor on which the substrates were mixed.

2.3. Biological material

The biological material was made of: the substrates (Saw dust and Corn cobs), the spawn. The different substrate types were cultivated in plastic bags (polyethylene) in an isolated house. A house was used because the cultivation method used was indoor method; the insect naturally present in the experimental house.

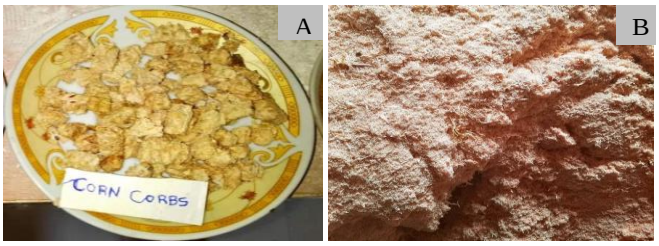

Figure 1. Different substrates used in mushroom production (A: corncob substrate, B: smooth powder sawdust substrate)

2.4. Methodology

The methodology used was adapted from the one of Mushroom Production Training and Research Center (MUPTAREC, 2012, 2021).

2.4.1. Preparation of different treatment substrate

Dry corn cobs were soaked in water on the 26th of May 2022 and left over night to absorbed moisture. They were removed from the water the following day in the morning so as to drain excess water. The sterilization process was carried out on the 27 of May 2022. The substrates (Saw dust; Corn cobs; Saw dust + corn cobs) were mixed in the ratio presented in Table 1. Substrates were soaked with 60 ml of water depending on the level of dryness until 60 % moisture content was achieved. The bagged substrate triplicates and tied up in 10×12 cm polyethylene were sterilized in autoclave for 15 minutes prior to inoculation at the temperature of 121°C to eliminate any microbial contamination and allowed to cool overnight.

Table 1. Different treatments of substrates used

Treatments	Substrate	Composition
T1	Saw dust	Saw dust, rice brand and $CaCO_3$ mixed in the ratio 32, 8 and 20 respectively.

T11		
T2 T22	Corn cobs	Corn cobs and $CaCO_3$ mixed in the ratio 40 and 20 respectively.
T3 T33	Saw dust + Corn cobs	Saw dust, corn cobs and $CaCO_3$ in the ratio 20, 20 and 20 respectively.

T1, T2, T3: treatment opened to insect visitors; T11, T22, T33: treatments protected from insects using gauze bag.

2.4.1.1. Preparation of treatment 1 (T1 and T11)

In preparing the substrate for treatment 1 and treatment 11, 32 kg of saw dust was measured using an electronic scale balance. Sawdust was the main common substrate for white oyster mushroom production in Cameroon [3]. It was placed on a clean cemented floor. The saw dust was of the fine type. Eight kg of rice bran was added on the heap. Rice bran contain cellulose, lignin, nitrogen [18]. The rice bran was to increase the porosity of the substrate. Calcium carbonate (white wash) of 20 g was slaked on a plywood by sprinkling water to it. The large crystals released much heat and became powder. The calcium carbonate was used to balance the pH and also to avoid infection by microorganisms. The combination was mixed with the hands to have a homogeneous mixture. Water was added and the substrate was mixed. After all these steps were done, hand sanitizer was used to sterilize the hand to avoid contamination. The substrate was then filled in black polythene bags with the hands. Each of the small black polythene bag contained 2 kg of the substrate to have the same weight and size. The bags were tied with a knot to ease it when it was untied for the next step of inoculation. These bags were ready for the sterilization process. 20 bags of 2 kg each were tied. Ten bags for treatment 1 and ten bags for treatment 11.

2.4.1.2. Preparation of treatment 2 (T2 and T22)

40 kg of corn cob was measured using a measuring scale and poured on a clean cemented floor. Calcium carbonate was slaked on a plywood by sprinkling water on it. The calcium carbonate evolved high amount of heat and broken down into powder and 20g of it was added to the heap of corn cob. This was mixed with the hands to obtain a homogeneous mixture. After it was mixed uniformly, water was added to the mixture to have a 60 % moisture content. It was examined when the substrate squeezed with the hands to see if water was going to drop from it. The hands were sanitized to kill microbial so as to avoid contamination. The substrate was then put into black polythene bags of 25×18 cm. The substrates were tied in 20 black polythene bags and each bag contained 2 kg of the substrate. 10 bags for treatment 2 and 10 bags for treatment 22.

2.4.1.3. Preparation of treatment 3 (T3 and T33)

The treatment contained a mixture of corn cob and saw dust. Sixteen kg of corn cob was measured and poured on a clean cemented surface. Another 16 kg of saw dust was added on the heap of corn cob. Eight kg of rice bran was also added to it. Calcium carbonate was slaked to powder on a plywood by sprinkling it with water. Twenty g of the calcium carbonate was added on the heap that contained the saw dust and corn cob. The heap was mixed uniformly

using sanitized hands. After the uniform mixture, water was added to it and it was mixed to have a 60 % moisture content. The substrate was tied in 20 different polythene bags and each bag contained 2 kg of the substrate. The mixture was tied by knot to ease the loosen process during inoculation. The moisture content was tested by squeezing the substrate with the hands to observe if water was to come out.

It was noted that: 20 bags of 2 kg each of each substrate was sterilized, inoculated and incubated; The corn cob was soaked in water for 6 hours before used so as to absorbed water; Blood meal was used in to boost fruiting and enable fast and healthy fruiting.

2.4.2. Sterilization of substrate

This is the process of destroying all microorganisms such as bacteria by heating. The substrates were sterilized with the used of an aluminum drum, heat, water and a big polythene bag (figure 2). Three large stones were arranged beside the farm house and 200 liters of aluminium drum was placed on the three stones. The size of the lid of the drum was reduced in circumference and four iron rods were attached to it and the side that had the rods was attached to the bottom of the drum, creating a space in between the bottom of the drum. The substrates were placed in the drum with corn cob at the base, the substrate that contained saw dust and corn cob at the middle and the saw dust at the top. The first layer of substrate at the bottom of the drum which was on top of the lid in the drum was placed at the wall of the drum. This created a space in the middle of the drum at the first layer. This space was to allow the passage of heat from below to the top of the drum. Old plastic bags were placed on the wall of the drum and to the top of the lid. This was to avoid any direct contact between the bags and the walls. This was done before placing the substrate in the drum. 40 litter of water was added in the drum before the substrates were placed in the drum. The drum surface was covered tightly with old nylon bags to avoid or reduce the out flow of heat. Fire was added under the drum using fire wood. The set up was sterilized by heating at 100 °C for 6 hours. This process was done in the evening from 6pm to 12 am. The drum containing the sterilized substrate was allowed to get cool while still on the fire stones. After all these steps, the substrate was ready for spawning and inoculation.

Figure 2. Some steps for sterilization of different substrates by heating for 6 hours (a: preparation of drum for sterilization, b: filling of substrates in the drum for sterilization, c: covering of substrates with polythene bags to retain heat during steaming)

2.4.3. Cultivation /Inoculation/ spawning

After proper hand sanitization to avoid contamination, cultivation was done by untying the polythene bags knots which was already sterilized and cooled down. The bags that contained the cooled substrates were placed on a clean

sterile floor which was dusted with calcium carbonate powder. The polythene bags were opened and bottles of matured spawn was used in the substrates. The bottles that contained the mature spawn were opened and half bottle of each the matured spawn was introduced into each of the substrate bags with a sterilized knife and mixed uniformly. These substrates were slightly pressed and tied. These bags were later placed on shelves built with timber board planks. Board were cut in to smaller sizes with space in between to allow free circulation of air, and to ease harvesting. These mixtures were then incubated (Incubation period was to allow the white oyster mycelium to colonized and grow through the substrate). The whole set was made of 60 bags. 30 bags made up of the 3 different substrates (T1, T2 and T3) which was placed on the upper side of the shelves. The other 30 bags made up of another 3 different treatments (T11, T22 and 33) was placed on the lower side of the shelf, arranged orderly according to the different substrates and each substrate type was given a name tagged on the substrate. The lower shelf was completely covered with a net. The room was kept closed for up to 3 weeks to prevent change in temperature, light and moisture and also to prevent attack by insects.

2.4.4. Incubation and observation

The inoculated bags were taken into the farm house for mycelium colonization/ growth at 25 °C – 30 °C and 90% relative humidity. After the inoculation period, the set up was kept for 3 weeks. This was to allow the colonization of the substrates by the mycelium. The mycelium grew through the substrates during this period. After the period of 3 weeks, some of the bags started fruiting pins heads that is immature fruiting bodies.

2.4.5. Fructification

It is the production of fruiting pins (immature mushroom bodies) right up to maturation of the fruiting bodies. The first harvesting was done on the 31st of July, 2022. After 25 days, substrate bags that were ramified completely were moved to mushroom growth chamber to enable sprouting and fructification. Tiny holes were created on the polyethylene bags using a wire. The polyethylene bags were opened and watered with 3 ml of water each using different syringes daily to avoid cross-contamination.

2.4.6. Harvesting

It is the collection of matured fruiting bodies for data analysis (figure 3). Fresh mushroom was harvested on maturation carefully by hand from the substrate bags after 31 days of inoculation. The first harvesting was done on the 31st of July, 2022. The harvested mushroom fruits were weighed, recorded and oven dried at 45 ^{o}C, to ensure that phyto-chemicals are not lost.

The matured fruiting bodies were harvested carefully to avoid destruction of the substrate using sanitized hands. All the fruiting bodies of a particular substrate bag were harvested at the same time. The matured white oyster mushrooms were harvested by holding them at the stipe, toward the lower level and were gradually pulled out of the substrate bag.

Watering was done by sprinkling water after each harvest to allowed fruiting for the next harvest that is to increase the relative humidity. Harvesting was done twice a week and the measurement of the growth parameters taken after each harvest and recorded. The same procedure was carried out for all treatments as well.

Figure 3. Harvesting, weighing using a kitchen scale and measuring of the diameter using measuring tape of *Pleurotus ostreatus* (a: matured mushroom ready for harvesting, b: harvested mushroom, c: weighing of mushroom using an electronic kitchen scale)

2.4.7. Data collection

Data was collected according to the method of Kumar et al. [19].

2.4.7.1. Growth parameters evaluated on mushroom

Mushroom growth was monitored through a series of stages and evaluated. These parameters include Spawn running time (in days), time for primordial initiation, pinhead formation to harvesting (in days), height of fruiting bodies (cm), number of fruiting bodies in a cluster, diameter of pileus of the fruiting bodies (cm), weight of individual fruiting bodies (g).

2.4.7.2. Effect of different substrates on mycelium growth

- Effect of different substrates on mycelium running time in spawn. Mycelium running rate (MRR) for each type of substrate was measured after the mycelium colony cross the shoulder of the packet. The linear length was measured at different points on the packet: MRR= L/N; Where L= Average length of the mycelium N= Number of days.

- Effect of different substrates on time from Stimulation to Primordial Initiation (days): Time required from stimulation to primordial initiation (days) were recorded.

- Effect of different substrates on time from Primordial Initiation to Harvest (days): Time required from primordial initiation to harvest (days) were recorded.

2.4.7.3. Effect of different substrates on yield contributing characters and yield

- Effect of different substrates on average height of fruiting bodies

The stalk length of the fruiting bodies was measured on the different substrates using a rope and a ruler and this gave different values.

- Effect of different substrates on average thickness of pileus.

The average thickness was measured by cutting through the pileus of different mushrooms using a blade and measured using a ruler.

- Average Number of fruiting Body/Packet: Number of well-developed fruiting body was recorded. Dry and pinheaded fruiting bodies were discarded but tiny fruiting bodies were included in counting.

- Average Weight of Individual Fruiting Body/Packet was calculated by dividing the total weight of fruiting body per packet by the total number of fruiting body per packet.

- Biological Yield (g): Biological yield per 500 g packet was measured by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion.

- Economic Yield: Economic yield per 500 g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard and dirty portion.

- Dry Yield: About 50 g of randomly selected mushroom sample was taken in a paper envelop and was weighed correctly. The mushroom was oven dried at 72 ºC temperature for 24 hours and weighed again. The weight of blank envelop was subtracted from both the weight. The dry yield was calculated using the following formula:

Dry Yield (g/500g) = Economic Yield X Dry weight of sample / Fresh weight of sample

- Drying of Mushrooms: The collected fruiting bodies of the mushroom were transferred to the laboratory. Therefore, data was collected on different parameter. After collection of the data, the fruiting bodies were dried in the sun separately treatment wise.

2.4.8. Study of the activity of insects on *Pleurotus ostreatus*

The frequency of insects was determined based on observations on the different parts of the fungi (Pileus, gills stipe) from the formation of the first fruiting pin, every two days, during each of the following daily time frame: 8-9 am, 9-10 am, 10-11 pm right up to 3-4 pm, from 26th May 2022 to 31st of August 2022 in Musang street-Bamenda. The observer passed once over each labelled substrate bag and for each of the four daily periods. At each passage, the identity of all species that visited the mushroom was recorded. The species were counted on the different part of the mushroom visited.

Three to five specimens of all species taxa found in the farm were captured with an aspirator and insect net. They were preserved in 70 % ethanol for subsequent taxonomic determination except those of the Lepidoptera which were wrapped in small dry papers following Borror and White (1991)[20] recommendations. All species encountered on the plant were registered and the cumulated results expressed in number of visits to determine their relative frequency in the anthophilous entomofauna of the mushroom.

In addition to the determination of the insects' frequency, direct observations of the foraging activity on Pileus, gills and stipe were made on species fauna in the experimental farm house and registered based on the preferred part of the fungi. The determination of the specimens was made in the biology laboratory of H.T.T.C Bambili of the University of Bamenda with the help of Prof Otiobo Nadine. During each daily period of observations, the temperature and relative humidity of the farmhouse were registered using a mobile thermo-hygrometer, every one hour.

2.4.9. Data Analysis

Data was analyzed using descriptive statistics (mean, standard deviation, percentage), correlation coefficient (r) for the study of the association between two variables, using Microsoft Excel 2010 and results were presented in tables, charts and graphs.

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3. Results

3.1. Effect of different substrates on mycelium growth

3.1.1. Effect of Different Substrates on Mycelium Running time in Spawn

Mycelium Running Rate per day (MRR) for each type of substrates was measured after the mycelium colony crossed the shoulder of the packet.

The highest running time was observed in T1 (24.20 ± 1.32 days) which was similar to T11 (24.00 ± 1.33 days) and statistically different from T2 (13.80± 1.70 days), *T*3 (18.40 ± 0.97days), T22 (13.90±1.29 days) and T33 (18.30 ± 1.06 days) treatment and the lowest mycelium running time of mycelium was observed in T2 (Figure 4).

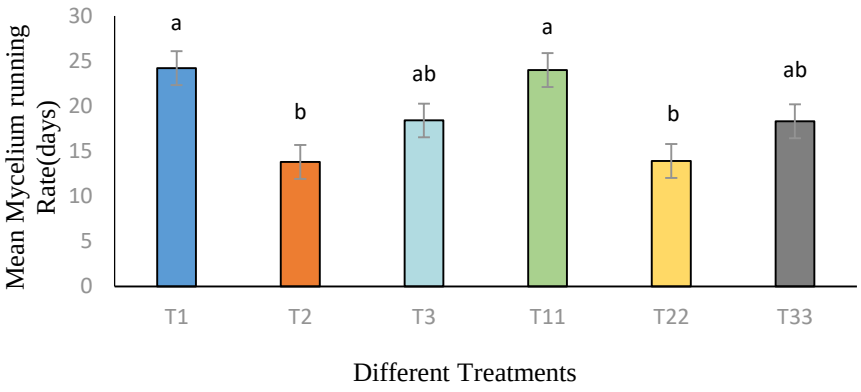

Figure 4. Mean of Mycelium Running time in Spawn at different Substrates after 21 days. Histograms with the same letter are not significantly different at $P \leq 0.05$

3.1.2. Effect of Different Substrates on Time from Stimulation to Primordial Initiation

The time from stimulation to primordial initiation ranged from 20 days to 50 days (Figure 5). The lowest time from stimulation to primordial initiation was observed in T2 and T22 (23.80 ± 1.87 days). The highest time from stimulation to primordial initiation was observed in T1 and T11 (46.30 ± 1.70 days). The time from stimulation to primordial initiation between T1, T2 and T3 were different as observed in figure 5.

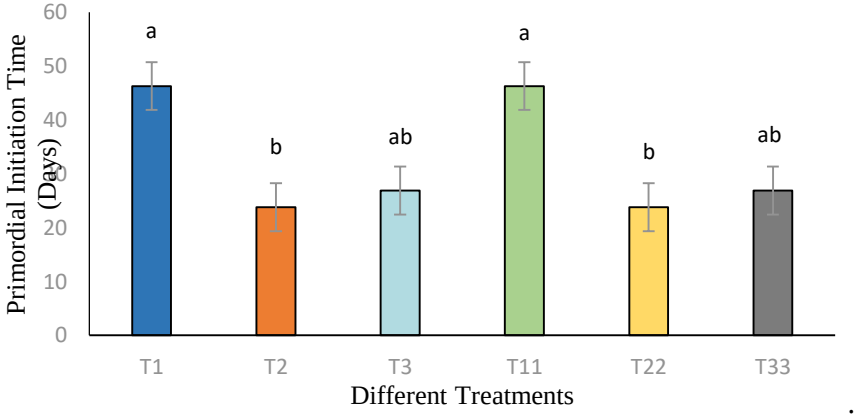

Figure 5. Primordial Initiation Time (Days) at different substrates Histograms with the same letter are not significantly different at $P \leq 0.05$

3.1.3. Effect of Different Substrates on Time from Primordial Initiation to Harvest (Days)

All the treatments were statistically similar but numerically vary from each other. Numerically the lowest time from primordial initiation to harvest was in the treatment T2 (5.40 ± 0.51days) followed by T22 (5.50 ± 0.53 day), T3 (6.70 ±0.67 days) and T33 (6.70 ± 2.17 days); and the highest time from primordial initiation to harvest was observed in the treatment T1 and T11 (6.80 ± 0.42 days) as shown in figure 6.

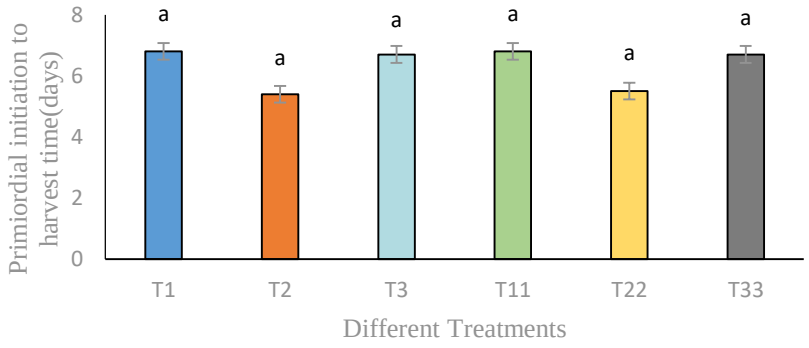

Figure 6. Effect of Different Substrates on Time from Primordial Initiation to Harvest (Days). Histograms with the same letter are not significantly different at $P \leq 0.05$

3.2. Effect of Different Substrate on Yield Contributing Characters and Yield

3.2.1. Effect of Different Substrates on Average height of fruiting bodies

The different substrates demonstrated several effect on the height of the fruiting bodies. The highest average length of stipe was observed in the treatment T3 (19.50 ±3.27 cm) followed by T22 (19.46 ± 2.13 cm) treatments and the lowest average length of stipe was in the treatment T2 (15.37 ± 0.70 cm). There was a significant difference between the heights of different substrates (figure 7).

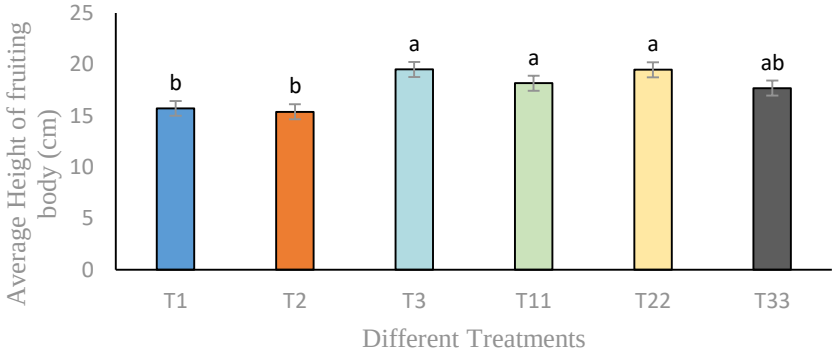

Figure 7. Effect of Different Substrates on Average height of fruiting. Histograms with the same letter are not significantly different at $P \leq 0.05$

3.2.2. Effect of Different Substrates on Average Thickness of Pileus

Effect of different substrates had great effect on average thickness of pileus. There was a significant difference between the thicknesses of pileus of the different substrates. The average thickness of the pileus in different

treatment ranged from 24.70 cm to 62.78 cm. The highest average thickness of the pileus was observed in substrate T22 (62.77 ± 4.28 cm) while substrate T1 had the lowest (24.70± 3.53 cm) (figure 8).

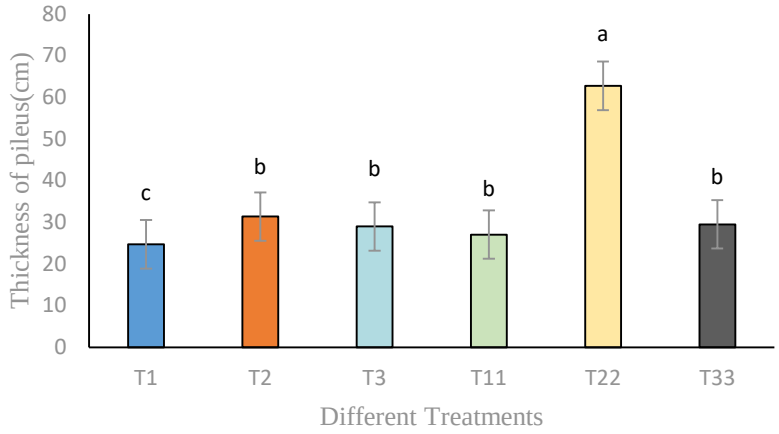

Figure 8. Effect of Different Substrates on Average Thickness of Pileus. Histograms with the same letter are not significantly different at $P \leq 0.05$

3.2.3. Effect of Different Substrates on Average number of Fruiting Body (fb)/Packet

The highest average number of fruiting body/packet was observed in the treatment T22 (6.70 ± 1.06 fb) and the lowest average number of fruiting body /packet was in the treatment T1 (5.10 ± 0.88 fb) followed by T11 (5.90 ± 0.57 fb). The other treatments were statistically and significantly varied over control in terms of average number of primordia/packet as shown in figure 9.

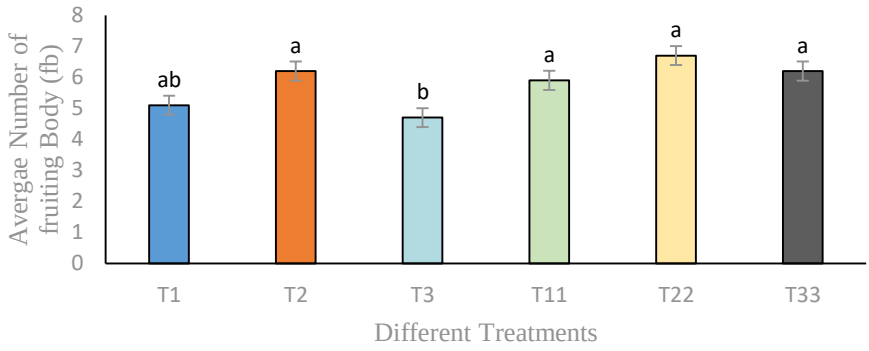

Figure 9. Effect of different substrates on Average number of fruiting bodies. Histograms with the same letter are not significantly different at $P \leq 0.05$

3.2.4. Effect of Different Substrates on Average Weight of Individual Fruiting Body (g)

Different substrates had great effect on average weight of individual fruiting body. The average weight of individual fruiting body in different treatment ranged from 114g to178g (figure 10). The highest average weight of individual fruiting body was observed in the treatment T33 (178 ± 85.7 g) followed by T3 (176 ± 84.3 g) treatment and the lowest average weight of individual fruiting body was in the treatment T1 (114 ± 55.1 g). The other treatments varied significantly over control in terms of average weight of individual fruiting body.

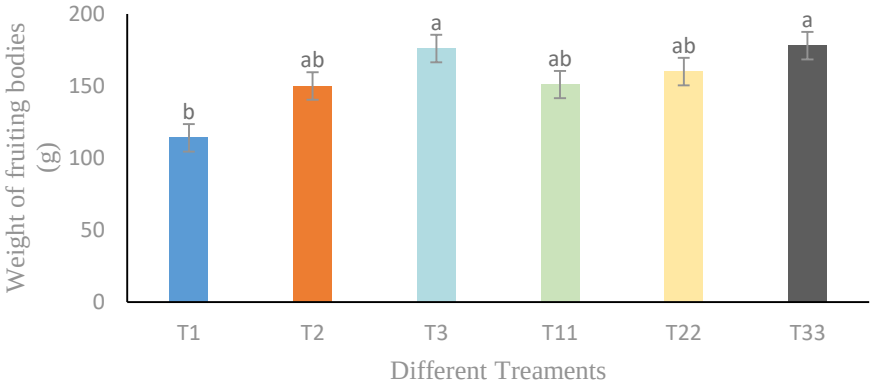

Figure 10. Different Substrates on Average Weight of Individual Fruiting Body (g). Histograms with the same letter are not significantly different at P≤ 0.05

3.2.5. Effect of Different Substrates on Biological Yield, Economic Yield (g) and Dry Yield

All the treatments were statistically similar but numerically vary with each other as shown in Table 2. Effect of different sawdust substrates had great effect on biological yield. Numerically the highest biological yield was counted under treatment T11 (279.3 g/packet) followed by T1 (275.1 g/packet) and the lowest biological yield was counted under T22 (247.1 g/packet). The other treatments varied significantly as compared with control.

Numerically the highest economic yield was recorded under treatment T11 (275.6 g/packet) followed by T1 (273.4 g/packet) and the lowest economic yield was counted under T2 (245.6 g/packet). The other treatments varied significantly over control.

The dry yield of the oyster mushroom, grown on different substrates responded significantly in terms of dry yield with supplement. Numerically the dry yield of mushroom was maximum under the treatment T11 (27.56 g/packet) followed by T1 (27.34 g/packet) treatment and the lowest dry yield was counted under T22 (24.63 g/packet). The other treatments varied significantly over control.

Table 2. Different Sawdust Substrates on Biological Yield, Economic Field (g) and Dry Yield

Substrate	Biological Yield (g)	Economic Yield (g)	Dry Yield (g)
T1	275.3[a]	273.4[a]	27.34[a]
T2	245.6[a]	247.8[a]	24.78[a]
T3	259.7[a]	261.3[a]	26.13[a]
T11	279.5[a]	275.6[a]	27.56[a]
T22	247.1[a]	246.3[a]	24.63[a]
T33	268.6[a]	264.2[a]	26.42[a]

Values represent mean of 6 different substrate groups and their effect on economic, biological and dry yield. Values not sharing common superscript letters (a-b) differ significantly; P<0.05.

3.3. Activities of species on *Pleurotus ostreatus*

3.3.1. Diversity of species on *Pleurotus ostreatus*

A total of 2464 visits of five species of Arthropods were collected on mushroom in Musang from 26th May 2022 to 31st of August 2022. The species were grouped into five Orders (Table 3).Each Order has one species. *Lycoriella mali* was the most frequent with 58.20 % of insect visits.The Araneae Order represented by the Family of the Arachnidae was the predator of other species. The number and percentage of visits of the different Arthropods are displayed in Table 3.

Table 3. Arthropods recorded on *Pleurotus ostreatus* in Musang from May 2022 to August 2022, number and percentage of visits by the different species

Order	Family	Genus, species	n	p (%)
Araneae	Arachnidae	gen. Sp.	79	3.21
Coleoptera	/	gen. Sp.	939	38.11
Diplopoda	/	millipede	5	0.2
Blattodea Diptera	cokroach	*Blata orientalis*	7	0.28
Diptera	Sciaridae	*Lycoriella mali*	1434	58.20
Total		5	2464	100

n: number of visits to the cultivated mushroom in 44 days; P: percentage of visits = (n / 2464) × 100.

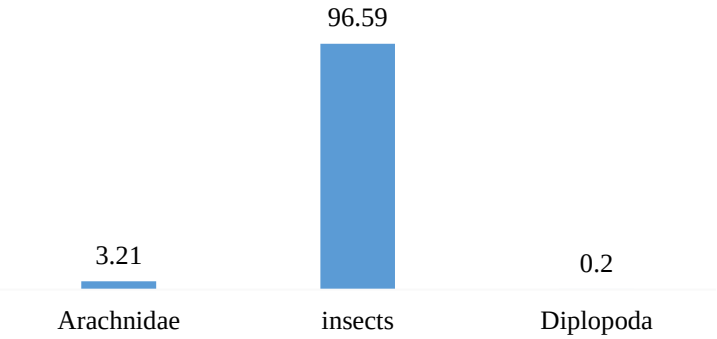

Figure 11. Percentage of different groups of Arthropods present on *Pleurotus ostreatus* from the farmhouse at Musang from the 26th of May to 31st of August 2022

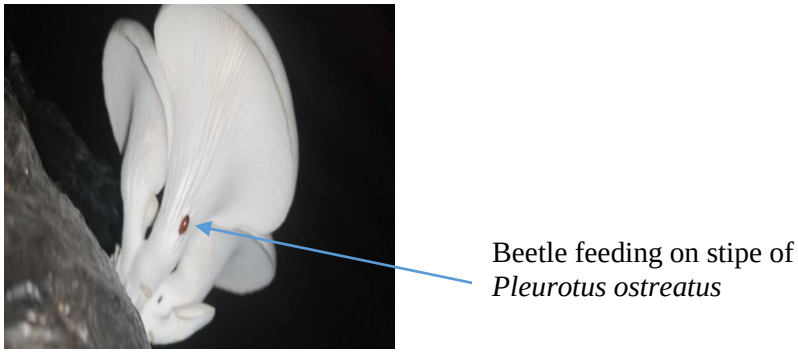

Figure 12. Insect species recorded on the stipe of *Pleurotus ostreatus*

3.3.2. Parts of the mushroom visited

Most of the species that visited the mushroom anchored or carried out their activities on different parts of the mushroom (figure 13). The various parts of the mushroom were visited at distinct significant levels, the pileus (57.6%), gills (24.2%) and stipe (18.1%)

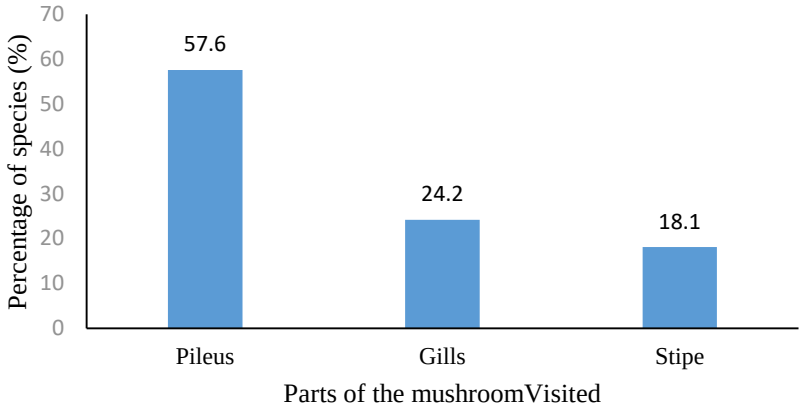

Figure 13. Percentage of species visiting mushroom according to mushroom parts

The different species were found feeding on pileus, stipe and lamella using mandibles. They are considered as voracious eaters that devour substrates, mycelium and matured mushroom Some were found grasping the pileus, others were cutting and destroying the pileus. The dipterian for example was found defecating worms on the stipes.

3.3.3. Rhythm of Visits According to the Daily Observation

Figure 14 shows that different organisms visit the mushroom throughout the period of the day from 8 a.m. to 3 p.m. The peak for visits was located in the morning between 8 am and 9 am. During this time slot, the average humidity was 35.16% and the average ambient temperature was 18.06 °C. These conditions could partly explain the higher frequencies of visits by these species during that time slot.

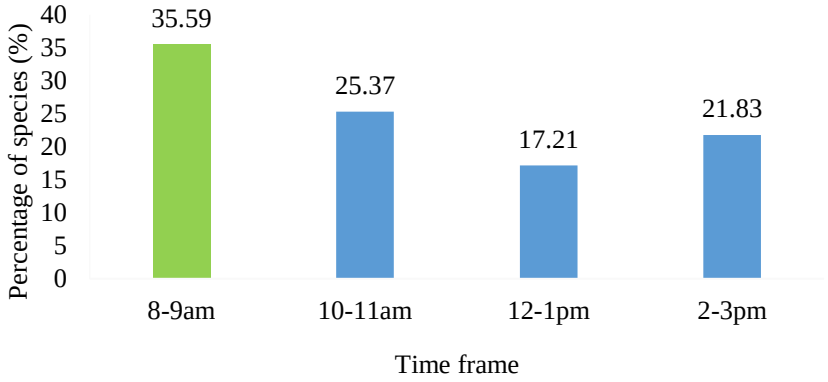

Figure 14. Percentage of species visiting according to daily time frame observation

3.3.4. Influence of Some Climatic Factors on species activities

Temperature and humidity seem to affect the activity of organisms in the mushroom. The correlation between temperature and number of visits was found negative and significant ($r = -0.98$; $df = 6$; $p < 0.05$). Regarding the

relative humidity of the air, our results revealed a positive and significant correlation ($r = 0.95$; $df = 6$; $p < 0.05$) as seen in figure 15 below.

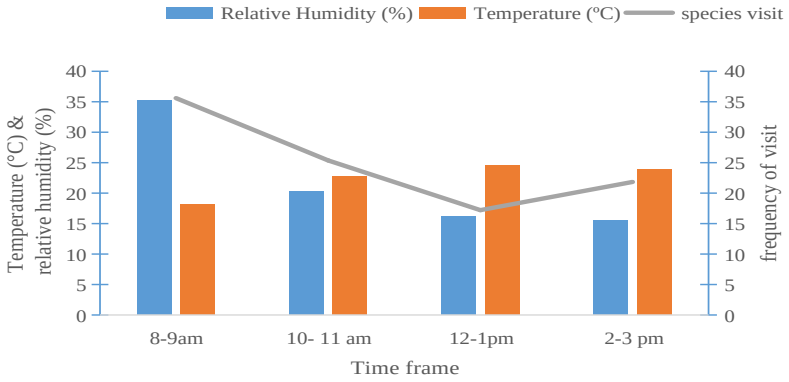

Figure 15. Frequency of species visiting mushroom farm according to temperature and relative humidity per time frame

4. Discussion

The result of the effect of different substrates on mycelium running time in spawn revealed that the time taken for the completion of spawn running was between 13 days to 24 days by the different substrates which correlates with results of Khan *et al.* [21] and Kalita *et al.* [22] who reported that time taken for completion of spawn running may require from 17 days to 22 days by use of different substrates. The effect of different substrates on time from stimulation to primordia initiation revealed the highest time from stimulation to primordia initiation was 47 days which is longer than that of Ahmed [23] who found out that *P. ostreatus* took 23-27 days for initiation of fruiting bodies. Quality of the substrate, experimentation area, abiotic factors are some of the conditions which may explain this difference.

Time from stimulation to primordia harvesting revealed that it took 5 to 7 days for harvesting. The findings of the present experiment are in conformity with Shah *et al.*, [24] who found that fruiting bodies of oyster mushroom became suitable for harvest within 3-6 day of primordia initiation in the spawn packet. Khan *et al.* [21] reported contrary to our study that after spawn running pinhead formation took 7-8 days and fruiting body formed after 3-5 days and sporocarps may be harvested after 10-12 days. The days required for first picking varied from 11.25 to 12 and the final picking complete from 42.25 to 43.50 days depending on different substrates.

Regarding the average height of fruiting bodies, the results revealed that mixed substrate yielded fruiting bodies with the highest weight and the weight yield of our study ranges from 15-18 cm. Results of our study varied with the findings of Sarker *et al.* [25] and Habib [26], who reported that the stipe length of *Pleurotus* spp. on different substrates varied from 1.93-2.97 cm and the diameter ranged from 0.74-1.05cm. The difference among the findings may be due to the difference in substrates and supplements used or the species varieties.

The average number of fruiting bodies range between 4-7 fruiting body per packet and differences between the substrate differed significantly with highest number observed in corncob substrate which varies with the results Yoshida *et al.* [27] who reported that the number of fruiting bodies was lower, but increased when the substrates were mixed with different supplements and consistent to the results of Amin *et al.* [2] who reported that the number

of primordia grown on different substrates differed significantly. Sarker [28] found that the number of primordia increased with the levels of supplement and continued up to a certain range.

Regarding the effect of different substrates on average weight of individual fruiting body, Sarker *et al.* [26] reported the individual weight of fruiting body ranged from 1.33-1.59 g, which was less similar to results obtained in this study. The results of Bhuyan [29] found significant effect of supplementation on the weight of fruiting body but he found comparatively lower weight of individual fruiting body ranged from 5.02-7.01 g, which may be due to environmental conditions or growing season. The difference among the findings may be due to the difference in substrates and supplements used or the species varieties. The humidity of the bags in which mushrooms were cultivated was favorable. The thickening of the mycelia in the bags, colonization of the bags was an indication for the bags to be opened for fruiting.

The biological yield of our experimental assay revealed a highest yield value of 279.3 g/packet which was observed in the substrate containing saw dust. Chowdhury *et al.* [30] examined the effects of adding different supplements to substrates for growing oyster mushrooms (*Pleurotus sajor-caju*) and found that adding 5% supplements gave the highest yield of oyster mushroom. Our results are in contrary with the results of Baysal *et al.* [31] who found that the highest yield of *P. ostreatus* was observed with the substrate composed of 20% rice husk in weight and in-line with Amin *et al.* [2] who found the highest biological yield 247.3 g/packet.

The highest economic yield recorded in this study was 275.6 g/packet which was observed in mushroom grown in sawdust substrate and is in line with the results of Bhuyan [29] who observed that the yield of *P. ostreatus* responded with the levels of supplements used with sawdust and increased with the level of supplementation. Payapanon *et al.* [32] mentioned that suitable amount of supplements added to rice straw medium maximized economic yield of oyster mushroom at optimum production cost. Sarker [28] found appreciable variations in economic yield was also observed at different levels of supplements under different substrate-supplement combinations.

The dry yield of the oyster mushroom, grown on different substrates responded significantly in terms of dry yield with supplement. Numerically the dry yield of mushroom was maximum under sawdust substrate with a yield of 27.56 g/ packet. The result of the present study corroborates with Ahmed [23] who observed significant effects of various substrates on diameter and length of stalk and diameter and thickness of pileus. Ahmed [23] also found that lower diameter of pileus produced the lowest yield and concluded that the diameter of pileus increased the quality and yield of mushroom and highest dry yield from mango sawdust.

From our observation, different parts of the mushroom were visited at distinct significant level. The pileus (57.6%), gills (24.2%) and stipe (18.1%).

The average temperature was 28.06 °C and the relative humidity of the study site was 20.56% seem to influence the growing of mushroom. Temperature too is needed for *P. ostreatus* to grow, particularly temperature of 25 ^{0}C heat to provide a cool respite for the growing mushroom [33]. *P. ostreatus* needs moisture; a regular supply of moisture will prolong the fruiting of fruiting pins (pinheads) and also the take up of moisture and nutrients helps the mushroom to grow well and produce succulent mushroom [34].

From the results obtained, we noticed that the Order Diptera (58.20%) were the most frequent insect on the *P. osreatus*, followed by Coleoptera (38.11%) and lastly by Blattodea (0.28%). This is similar with the study of Cevik [35] who reported that there are three fly pest groups most commonly encountered in mushroom-producing areas. This was due to the following reasons. Firstly, Diptera, Coleoptera and Blattodea are extremely abundant in most ecosystems, they compromise at least one third of all insect biomass and may equal the biomass of the human population, with this they are found everywhere [36]. Again, some Diptera, Coleoptera and Blattodea are predators or parasitic insects which cause the browning of the stipe, some also feed on the pileus, lamella and stipe of the mushroom as indicated Hockings [37]. In this case, increase number of insect's species correspond with higher humidity during the early morning periods. As time goes on (lower humidity) from the morning periods, insect species also reduce.

The productivity of *P. ostreatus* was observed to have less effect from insects nor their frequency. More so, it was observed that the insects played a negative role by destroying mushrooms once they started fruiting and when they matured thereby reducing the quantity of edible mushroom. Therefore, from observation from this work, there is no correlation between insects visit with frequency and yield of edible mushroom but in terms of edible mushroom, the quantity and quality of mushroom dropped when insects started visiting the farm.

5. Conclusion

The study has shown that oyster mushroom could be cultivated using saw dust and corn cobs. Saw dust substrate had the highest yield and the most suitable for oyster mushroom cultivation followed by a combination of saw dust and corn cobs. Still from this findings, it was realized that, different species of arthropods were observed on the different part of the mushroom. That is Arachnidae, Insecta and Diplopoda. In terms of edible mushroom, the quality and quantity dropped when insects start visiting the farm.

Sawdust and Corn Cobs are relatively abundant in rural communities in the study area where resource poor farmers reside and it can therefore be used to cultivate *P. ostreatus*, providing a highly profitable agribusiness that produces not only nutritious and medicinal food products from different substrates, but also helps to dispose them in the environment in a friendly manner and conserving mushroom biodiversity in the forest.

6. Recommendations

(i) Farmers are recommended to use saw dust and corn cobs in the ratio 4:2 respectively in the cultivation of mushroom.

(ii) Saw dust can equally be used without any supplement as this will give better yields at times. Therefore, farmers are recommended to use saw dust.

(iii) The government should organize seminars in order to educate farmers practicing mushroom cultivation on the different techniques used to cultivate the different species of mushrooms.

(iv) Farmers should practice subsistence mushroom cultivation wherein they make use of plant waste in order to avoid unnecessary expenditure.

Declarations

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This study did not receive any grant from funding agencies in the public or not-for-profit sectors.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

The authors declare that they consented to the publication of this study.

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